American Thoracic Society
MEDICAL SECTION OF THE AMERICAN LUNG ASSOCIATION

Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide in Adults and Children—1999

THIS OFFICIAL STATEMENT OF THE AMERICAN THORACIC SOCIETY WAS ADOPTED BY THE ATS BOARD OF DIRECTORS, JULY 1999

Airway inflammation is a central process in asthma and other lung diseases (1). The direct sampling of airway cells and mediators can be achieved by invasive techniques such as bronchoscopy with lavage and biopsy, or by the analysis of induced sputum. However, exhaled breath contains volatile mediators such as NO (2), CO (3–5), ethane, and pentane (6–8), but also nonvolatile substances in the liquid phase of exhalate, termed breath condensate, for example, hydrogen peroxide (9–12). The noninvasive nature of the measurement of exhaled mediators makes them ideally suited for the serial monitoring of patients.

The presence of endogenous nitric oxide (NO) in exhaled breath of animals and humans was first described in 1991 (2). Soon after, several publications reported high levels of orally exhaled NO in subjects with asthma as compared with unaffected subjects (13–18) and a fall in these high levels after treatment with corticosteroids (15, 19, 20). Similar findings have been described in the pediatric age group (21–24). In patients with chronic obstructive pulmonary disease (COPD), exhaled NO has been reported to be high in exacerbations compared with stable patients (25). Other diseases associated with high exhaled NO include bronchiectasis in one study (26) but not in another (27), viral respiratory tract infections (28, 29), systemic lupus erythematosis (30), liver cirrhosis (31–34), and acute lung allograft rejection (35). Low levels of exhaled NO have been described in cystic fibrosis (21, 36–39), human immunodeficiency virus (HIV) infection (40), and pulmonary hypertension (41, 42). Exhaled NO has been shown to correlate with other parameters in mild asthma, for example, induced sputum eosinophilia (43) and bronchial reactivity (44) in non-steroid-treated subjects.

Nasal nitric oxide concentrations are high relative to the lower respiratory tract in humans (18, 45), with the highest levels reported in the paranasal sinuses (46, 47). Nasal NO may have physiologic roles, for example, preserving sinus sterility (48) and modulating ciliary motility (49). Nasal NO concentration has been proposed as a surrogate marker of nasal inflammation such as occurs in allergic rhinitis (50–54). In contrast, subjects with immotile cilia syndromes and cystic fibrosis have low nasal NO (55, 56).

Although there are numerous publications on exhaled NO, the study of exhaled and nasal NO measurement has been characterized by a variation in published exhaled NO levels in health and disease, much of which is attributable to the lack of a standardized technique of measurement. Many investigators have employed or recommended different methodologies for exhaled NO measurement (57–64); there is little published about nasal NO measurement techniques.

In order for the field of NO measurement to advance, it was felt that an international consensus on the appropriate measurement techniques was required as a basis for the collection of normative data and the application of standardized techniques to measurements made in various disease states. A taskforce of the European Respiratory Society published European recommendations in 1997 (58).

The recommendations in this document were formulated by international investigators in the field of exhaled and nasal nitric oxide, at a workshop sponsored by the American Thoracic Society (ATS)/American Lung Association (Toronto, Canada, May 1998). Also attending as committee members to assist in the technical recommendations were scientists from nitric oxide analyzer manufacturers: Aerocrine, Eco Physics, Sensor Medics, and Sievers Instruments. The workshop consisted of five committees: adult online exhaled nitric oxide measurement, offline exhaled nitric oxide measurement, pediatric online exhaled nitric oxide measurement, nasal nitric oxide measurement, and technical recommendations. The initial draft of the document was prepared during the workshop by the moderators of each of the committees and presented to the entire forum at the last session of the workshop. Modifications were made subsequent to frequent communication between the members of the breakout sessions, and in light of comments from external reviewers. The revised document was circulated to all workshop members for review and presented to the ATS board of directors in July 1999.

The standardization of techniques opens the way for the collection by numerous centers of comparable data from normal subjects and those with disease states. The workshop participants felt that adequate knowledge and experience were available to warrant presentation of the guidelines that follow. The document is divided into a general section that deals with aspects common to all sections, followed by those dealing with adult online and offline measurement, pediatric measurement, nasal NO measurement, and technical aspects of NO analysis. Wherever possible, the small number of recommendations are based on published material including abstracts as referenced; in the absence of clear data, we have relied on the experience of participants in the workshop. Where aspects concerning exhaled NO measurement are undetermined, this has been clearly stated in text.

While these recommendations will allow uniformity of measurement techniques in future studies, this document does not

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intend to invalidate previous or ongoing studies that have employed other techniques. Wherever practical, investigators are encouraged to include the recommended method in addition to the measurement techniques with which they are familiar, so that the knowledge concerning the recommended methods will increase. This will allow future modifications of these recommendations to be made on scientific grounds.

1. GENERAL ASPECTS OF EXHALED AND NASAL NITRIC OXIDE MEASUREMENT

Requirements for the Clinical Use of Exhaled NO Measurements

At present, exhaled and nasal NO measurements have been performed in the research setting. *Online measurement* refers to exhaled NO testing with a real-time display of exhaled NO breath profiles, whereas *offline testing* refers to collection of exhalate into suitable receptacles for delayed analysis. The use of exhaled NO measurement as a clinical tool requires the adoption of a standardized measurement technique followed by collection of normative data in all age groups. The achievement of a consensus as detailed in this document will enable an international multicenter collaborative study using standardized techniques. Ideally, there should be interinstitutional agreement of mean exhaled NO within 5% for each age group. Also, clinical indications for the measurement of exhaled NO should be validated.

Standardization of Exhaled NO Terminology and Units

Nomenclature and symbols used in articles reporting exhaled NO have been variable. The following guidelines have been formulated to bring this field of study in line with standard physiological nomenclature.

Online measurement. The fractional exhaled NO concentration (F_{ENO}) is expressed in parts per billion (ppb), which is equivalent to nanoliters per liter (nl/L). The exhalation flow rate employed for a particular test can be expressed as a subscript of the flow in liter/sec, for example, $F_{ENO,0.05}$. *Expired* and *exhaled* are both denoted by E, and *inspired* by I, in qualification of the test results, for example, F_{ENO} and F_{INO}.

NO output represents the rate, that is, the amount of NO exhaled per unit time, and is denoted by $\dot{V}_{NO}$. It is calculated from the product of NO concentration in nanoliters per liter and expiratory flow rate in liters per minute, corrected to BTPS.

$$\underset{\text{nl/min}}{\dot{V}_{NO}} = \underset{\text{nl/L}}{[NO]} \times \underset{\text{L/min}}{\text{airflow rate}}$$

Terms such as "NO release," "NO excretion," "NO secretion," and "NO production" are to be discouraged when referring to $\dot{V}_{NO}$.

Offline NO collection. F_{ENO} refers to the fractional NO concentration in exhalate from a vital capacity collection. If the exhalation is at a constant flow, this should be added as a subscript, for example, $F_{ENO,0.35}$, with the flow rate in liters per second.

Nasal NO. The fractional concentration of nasal NO is termed nasal F_{ENO}. Nasal NO output is the rate of nasal NO exhaled and should be represented as nasal $\dot{V}_{NO}$.

General Principles Regarding Exhaled NO Measurement

Source of exhaled nitric oxide. Current thinking is that NO is formed in both the upper and lower respiratory tract (55, 65–71) and diffuses into the lumen by gaseous diffusion down a concentration gradient, thus conditioning exhaled gas with NO (60, 72, 73). Alveolar NO is very low owing to avid uptake by hemoglobin in pulmonary capillary blood (68, 74). Although gastric NO levels are very high (75), this does not appear to contaminate exhaled NO (75), probably owing to closed upper and lower esophageal sphincters.

Nasal NO contamination. Nasal NO can accumulate to high concentrations relative to the lower respiratory tract (46, 47, 55, 76–79). The issue of the relative contribution of nasal NO to exhaled NO has been addressed in many publications (18, 45, 46, 57, 59, 62, 76, 78, 80). Accordingly, techniques that aim to sample lower respiratory NO should prevent contamination of the sample with nasal NO (57, 59).

Ambient nitric oxide. As environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO. The ways of achieving this are method specific and are discussed in each section. Notwithstanding which technique is employed, ambient NO at the time of each test should be recorded.

Expiratory flow rate dependence. Exhaled NO concentrations from the lower respiratory tract exhibit significant expiratory flow dependence (59, 81) and the same holds for the nasal cavity (82, 83). This variation in exhaled NO has been attributed to faster flows minimizing the transit time of alveolar gas in the airway. The rate of NO output, however, is greater at higher flow rates, but not in direct proportion; this is analogous to respiratory heat loss (59). In view of this flow dependency, the use of constant expiratory flow rates is emphasized in standardized techniques.

Breathhold. Breathhold results in NO accumulation in the nasal cavity, lower airway, and probably in the oropharynx and this results in NO peaks in the exhalation profiles of NO versus time (46, 55, 61, 66, 84). For this reason, the use of breathhold is discouraged in the standardized techniques described in this document.

Patient Factors Influencing Exhaled NO Values

The following factors are pertinent to online and offline exhaled NO measurement in both adults and children. Some of the factors mentioned below may affect nasal NO levels and are discussed separately in Section 5.

Age/sex. In adults, there is no consistent relationship between exhaled NO level and age, sex (85), menstrual cycle, or pregnancy (63, 86) but these patient characteristics should be recorded at the time of measurement. One study reported that, in children 7–13 yr of age, F_{ENO} increased with age (87).

Respiratory maneuvers. Because spirometric maneuvers have been shown to transiently reduce exhaled NO levels (88, 89), it is recommended that NO analysis be performed before spirometry. The same stipulation applies to other taxing respiratory maneuvers, unless these can be shown not to influence exhaled NO. The exhaled NO maneuver itself does not appear to affect plateau exhaled NO levels (89).

Airway caliber. It has been demonstrated that exhaled NO levels may vary with the degree of airway obstruction (20, 90), or after bronchodilatation (89, 91, 92), perhaps owing to a mechanical effect on NO output. Accordingly, the time of last bronchodilator administration should be recorded.

Food and beverages. There are insufficient data in the literature to make a firm recommendation concerning whether or for how long patients should refrain from eating and drinking before NO analysis. An increase in exhaled NO has been found after the ingestion of nitrate- or nitrate-containing foods, such as lettuce (with a maximum effect 2 h after ingestion) (93), and drinking of water may lead to transiently altered NO levels (94). It is possible that a mouthwash may reduce the effect of nitrate-containing foods (93). Until more is known, it is pru-

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dent when possible to refrain from eating and drinking for 1 h before to exhaled NO measurement, and to question patients about recent food intake. Alcohol ingestion reduces exhaled NO in patients with asthma and in unaffected subjects (95, 96).

Circadian rhythm. Studies are in progress to examine the effect of circadian rhythm on exhaled NO, so it is uncertain whether measurements need to be standardized for time of day (97, 98). It is therefore prudent to perform serial NO measurements at the same time of the day when possible and to always record the time.

Smoking. Chronically reduced levels of exhaled NO have been demonstrated in cigarette smokers in addition to acute effects immediately after cigarette smoking (17, 99–101). Subjects should not smoke in the hour before the study and short- and long-term active and passive smoking history should be recorded.

Infection. Upper and lower respiratory tract infections may lead to increased levels of exhaled NO (28, 29). Therefore exhaled NO measurements should be deferred until recovery if possible or the infection should be remarked on in the record.

Other factors. Manipulation of physiological parameters has been shown to affect exhaled NO. Changing pulmonary blood flow has no effect in humans (102), but hypoxia decreases exhaled NO (68, 103). The application of positive end-expiratory pressure (PEEP) has been shown to increase exhaled NO in animals (104–106), but airway pressure in humans does not affect exhaled NO plateau levels (59, 81, 94). Many studies have examined the effect of exercise on exhaled NO and nasal NO (41, 80, 84, 107–114). During exercise, exhaled NO and nasal NO fall while NO output increases. The duration of this effect after exercise is unknown. It is therefore prudent to avoid strenuous exercise for 1 h before the measurement.

Medications and Exhaled NO

The potential effect of drugs on NO cannot be excluded, and so all current medication and time administered should be recorded. Exhaled nitric oxide falls after treatment with inhaled or oral corticosteroids in asthmatic subjects (15, 16, 19, 23, 115–117) and after inhalation NO synthase inhibitors (118). The effect of other anti-inflammatory agents is not yet published. NO donor drugs (119) and oral, inhaled, and intravenous L-arginine (64, 120) increase exhaled NO and nasal NO (121). Even if a certain medication does not affect NO production, it might affect the apparent level of NO through other mechanisms such as changes in airway caliber (89–92, 122).

2. RECOMMENDATIONS FOR A STANDARDIZED PROCEDURE FOR THE ONLINE MEASUREMENT OF EXHALED NITRIC OXIDE IN ADULTS

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Online methods refer to exhalations where the expirate is continuously sampled by the NO analyzer, and the resultant NO profile versus time or exhaled volume, together with other exhalation variables (e.g., airway flow and/or pressure) are captured and displayed in real time. This enables the test administrator to monitor the exhalation to ensure conformation to the required flow and pressure parameters and the achievement of an adequate NO plateau. Suboptimal exhalations can be immediately identified and discarded. The online method requires more stringent analyzer specifications (*see* Section 6).

Recommended Technique for Online Adult Exhaled NO Measurement

Inspired gas source. Although there is evidence that ambient NO levels do not affect the single-breath plateau levels of exhaled NO (59), the use of NO-free air (containing < 5 ppb) for inhalation is preferable. When inhaling gas containing high levels of NO, an early NO peak is observed in the exhaled NO profile versus time, probably due to the ambient NO present in the instrument and patient dead space (59). This peak takes time to wash out, which increases the time elapsed until a plateau is reached, with resulting prolongation of the exhalation. In all studies, it is advisable to record ambient levels of NO.

Inhalation procedure. The patient should be seated comfortably, with the mouthpiece at the proper height and position. A nose clip should not be used, because this may allow nasal NO to accumulate, and promote leakage of this NO via the posterior nasopharynx. However, if subjects cannot avoid nasal inspiration (seen as an early expiratory peak) or nasal exhalation, a nose clip may be used. The patient inserts the mouthpiece and inhales through the mouth to total lung capacity (TLC) over 2–3 s, and then exhales immediately, as breathholding may affect exhaled NO. TLC is recommended, as this is the most constant point in the respiratory cycle and patients accustomed to spirometry are familiar with inhaling to this volume.

Exhalation procedure. Two factors are critical in ensuring reproducible and standardized measurements of lower respiratory tract exhaled NO.

1. Exclusion of nasal nitric oxide: Exclusion of nasal NO is important in view of the high nasal NO levels relative to the lower respiratory tract (18, 46, 55, 76–78, 82). This nasal NO can enter the oral expiratory air via the posterior nasopharynx. Closure of the velopharyngeal aperture during exhalation is one way to minimize nasal NO leakage. This can be achieved by exhaling against an expiratory resistance with a positive mouthpiece pressure (57, 59). It is common practice to display pressure or expiratory flow to the subject, who is requested to maintain these within a certain range. The procedure causes velum closure as validated by nasal CO_2 measurement (59) and nasal argon insufflation studies (57). The resultant mouthpiece pressure should be at least 5 cm H_2O to ensure velum closure and exclude contamination of the expirate with nasal NO. However, a pressure greater than 20 cm H_2O should be avoided as this may be uncomfortable for patients to maintain. One simple apparatus for the restricted breath technique is shown diagrammatically in Figure 1. In addition to the restricted exhalation method, another acceptable technique is continuous nasal aspiration (123, 124), which reduces nasal NO leakage either by removing NO and thus preventing the accumulation of nasal NO, and/or by itself causing velopharyngeal closure. A third published method uses the inflation of a balloon in the posterior nasopharynx (46, 76). However, this is not practical for routine clinical use.

2. Standardization of exhalation flow rate: Exhaled NO plateau values vary considerably with exhalation flow rate owing to variation of airway NO diffusion with transit time in the airway (59, 81, 125). Therefore, standardization of exhalation flow is critical for obtaining reproducible measurements. Low flow rates (< 0.1 L/s) amplify the measured NO concentrations and are felt to aid in discriminating among subjects (69, 73). In addition, the resulting higher exhaled NO values avoid measurement close to the detection limits of current NO analyzers. On the negative side, lower flow rates result in longer exhalation times to reach an NO plateau (59) and the prolongation of the exhalation may be uncomfortable for some pa-

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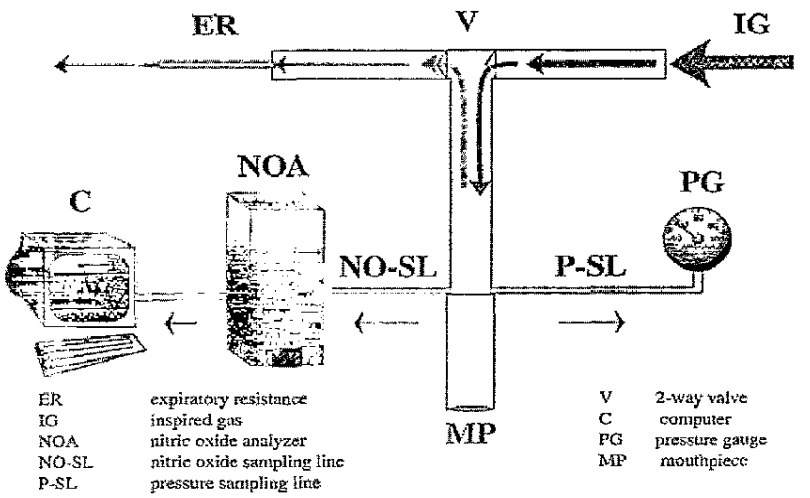

Figure 1. Diagram of one configuration for the breathing circuit employed in the restricted breath technique. *See text* for explanation of technique.

tients with severe disease. Low flow rates are also associated with a decreased NO output (59, 81).

Recommended Expiratory Flow Rate

A flow rate of 0.05 L/s (BTPS) was thought, on the basis of current knowledge, to be a reasonable compromise between measurement sensitivity and patient comfort. However, exhaled NO measurement can be performed at higher or lower flow rates if this is desirable in certain situations. In all cases, however, the expiration flow should be clearly recorded and reported in any publications.

A constant expiratory flow can be achieved in different ways. One commonly used method to achieve a constant expiratory flow is to display a target mouthpiece pressure or flow to the subject (e.g., using a gauge or computer display) while the subject exhales via a fixed expiratory resistance (59, 81). The constant pressure and therefore flow is achieved by biofeedback of pressure or flow parameters to the subject, who maintains these parameters within specified limits.

Exhalation pressure does not affect NO plateau measurements (59, 81, 94), and so individual investigators may select pressures between 5 and 20 cm H_2O, with the appropriate expiratory resistance to achieve the desired flow.

With biofeedback of expiratory pressure or flow, most subjects are able to maintain low flow rates that vary little from the desired target. In general, an exhalation is deemed adequate if the mean exhalation flow rate is 0.05 L/s (± 10%) during the time of the NO plateau generation and the instantaneous flow is not less than 0.045 L/s or greater than 0.055 L/s at any time during the exhalation. If it is not possible to keep within these values, the results should still be recorded and the failure to achieve this flow criterion noted in the record.

Recent theoretical considerations suggest that it is possible to derive other parameters such as an airway diffusion rate, effective mucosal surface concentration, and alveolar NO levels by the measurement of exhaled NO at multiple flow rates (69–71, 73). However, the additional contribution of exhaled NO measurement at multiple flows relative to measurement at one flow alone is of unproven value at this stage to justify recommendation for general application, and it should therefore remain a research tool at present.

The Interpretation of NO Single-Breath Profiles

Constant flow exhalations, however achieved, result in a single-breath NO profile (exhaled NO versus time plot) that consists of a washout phase followed by an NO plateau, which is usually reproducible and flat (Figure 2) but may slope up or down (Figure 3). The washout phase is sometimes followed by an early NO peak before the plateau (Figure 4). This peak may be derived from the nasal cavity if the subject inhales through the nose or the velum is open initially as the exhalation starts. In addition, NO in the inhaled air source (*see* INSPIRED GAS SOURCE, *above*), and NO accumulating in the oral cavity and lower airway if the subject pauses at TLC, may also generate an early peak. Early peaks are ignored, and only NO plateaus are interpreted.

The duration of exhalation must be sufficient (at least 6 s) to obtain a plateau in the NO versus time profile of at least 3 s; the plateau starts at point A and ends at point B and may be flat, up sloping, or down sloping (*see* Figure 3). Once a 3-s plateau is achieved, there is no reason to continue the exhalation.

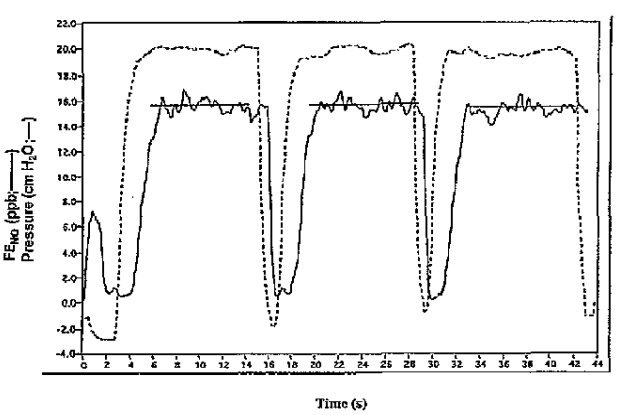

Figure 2. NO concentration (ppb) and airway opening pressure versus time for three separate exhalations by the same subject, showing reproducible profiles and plateaus.

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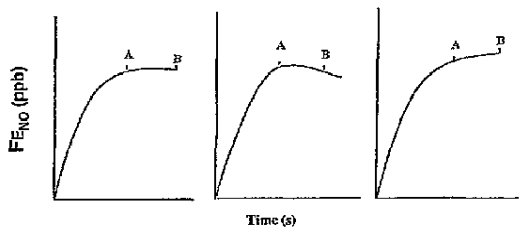

Figure 3. Schematic diagram of exhaled NO profiles, showing (from *left* to *right*) horizontal, downsloping, and upsloping NO plateaus with the start (*A*) and the end (*B*) of an NO plateau as defined in text.

The plateau is defined as the first portion of the NO versus time profile where A − B or B − A, related to the smaller of A and B, is < 10%. Also, at no time between points A and B should the NO values be greater than the NO value at A or B (Figure 3). For exhaled NO value < 5 ppb, the 10% plateau criterion may be difficult to fulfill; in such cases, a change of 1 ppb or less between points A and B is an acceptable plateau. Online electronic analysis of NO profiles allows automatic identification of valid NO plateaus according to these criteria. At the recommended flow of 0.05 L/s, plateaus are usually flat and clearly discernible (Figure 2).

Repeated reproducible exhalations should be performed, resulting in three NO plateau values that agree within 10% of the mean value. Exhaled NO is then calculated as the mean of these three values (Figure 2). At least 30 s of relaxed tidal breathing off the NO measurement circuit should elapse between exhalations, to allow subjects to rest. Care must be taken not to exhaust the patient when repeated exhalations are unsatisfactory.

3. RECOMMENDATIONS FOR OFFLINE MEASUREMENT OF EXHALED NITRIC OXIDE IN ADULTS

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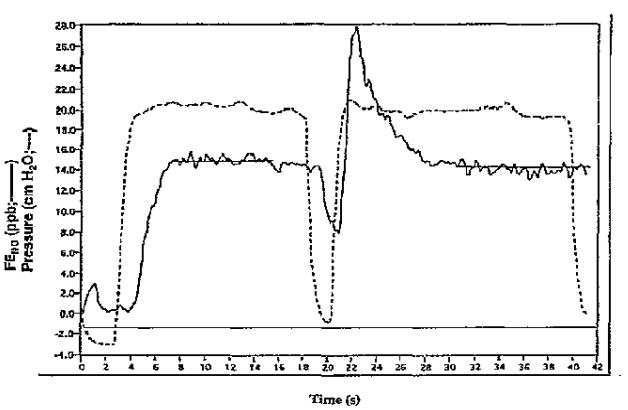

Figure 4. NO concentration and airway opening pressure versus time. The left-hand trace was performed with an oral inspiration of gas containing < 5 ppb NO. The right-hand trace shows an early peak that is generated by asking the subject to inhale nasally. This fills the conducting airways with nasal NO, which is then exhaled. A similar peak can be produced by inhaling ambient NO. The NO plateau is essentially unaltered once the early peak has washed out.

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Nitric oxide determinations can be made from exhaled gas collected in a reservoir and subsequently analyzed for NO concentrations. Several groups have used reservoir collection techniques to study nitric oxide in humans and, while the absolute FE_{NO} values vary in different reports, the relative changes in FE_{NO} in disease states observed by these authors are consistent with the findings reported by authors using online methods (15, 16, 18, 84, 88, 126).

Advantages and Disadvantages of Offline Collection

As compared with online techniques, offline collection offers (*1*) the potential for expirate collection at sites remote from the analyzer, (*2*) independence from analyzer response times, and (*3*) more efficient use of the analyzer, as gas may be collected from several patients simultaneously and less analyzer time per patient is required.

Potential problems with offline methods include (*1*) contamination with gas not derived from the lower airway, (*2*) error introduced by sample storage, and (*3*) an inability to allow for instantaneous feedback and assessment of technique. Recommendations regarding the standardization of expirate collection and storage for the offline measurement of FE_{NO} are presented in the following sections.

Procedures for Collection of the Sample

For ambulatory patients, it is recommended that gas for FE_{NO} be collected by asking the patient to inhale orally to TLC and then immediately perform a slow vital capacity maneuver against an expiratory resistance into a reservoir bag without a breathhold. The reservoir is sealed and subsequently analyzed for FE_{NO}. Details pertaining to this maneuver and to the equipment needed for this measurement are presented in the following sections, and one simple apparatus for the offline collection is shown in Figure 5.

Inspired Air

When measuring exhaled NO offline, evidence provided by several groups demonstrates that high inhaled concentrations of NO affect FE_{NO} measurements; determinations made with air containing more than 20–40 ppb NO are significantly higher than values obtained when subjects are inspiring air containing negligible amounts of NO (59, 88, 127). It is likely that this effect results from contamination of dead space gas in the collection device with high-NO ambient gas. As spontaneously occurring indoor ambient NO concentrations in urban centers may reach several hundred parts per billion, it is critical to actively control the NO concentration of the inspired gas when the collected sample contains dead space gas. This can be accomplished by asking the subjects to breathe from a source of low-NO air or through an NO scrubbing filter for 15 s (or a minimum of two tidal breaths) before the collection of the expirate (88).

Expiratory Flow and Oropharyngeal Pressure

It is known that the concentration of NO recovered in the expirate decreases with collection at higher flow rates (59, 81). This change in the recovered FE_{NO} likely represents the dynamic equilibrium between the production of NO (or its release) in the airway and the diffusion of NO into the gas flowing through the airway (128).

The Recommended Flow Rate

During offline collections, the flow rate of the exhalate must be known. For collection of the entire vital capacity, an expi-

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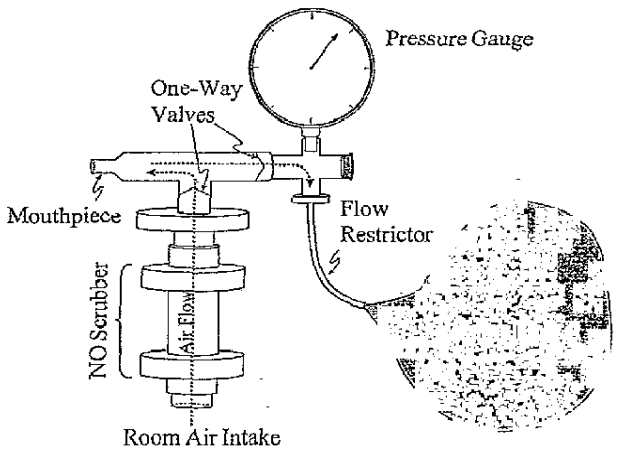

Figure 5. A diagram of an apparatus used in the offline collection of exhaled NO. *See text* for explanation.

ratory flow rate of 0.350 L/s is recommended, with flow not less than 0.315 L/s and not exceeding 0.385 L/s at all times during the exhalation. This flow rate was selected because the FE_{NO} is less sensitive to flow in this flow range, and also to allow most patients to expire their vital capacity over less than 20 s.

Nasal NO Contamination

As the concentration of NO in the nasopharynx may be high relative to that recovered in the lower airway, the nasopharyngeal gas must be excluded from the expirate collected by an offline technique. The two methods commonly used to isolate lower airway gas from that produced in the upper airway are (*1*) nasal suction and (*2*) exhalation against a resistance, thereby elevating the soft palate. Nasal suction is a more invasive and uncomfortable technique. Maintenance of an oropharyngeal pressure of at least 5 cm H_2O during exhalation minimizes nasal contamination of the sample by ensuring closure of the soft palate (57, 59). This oropharyngeal pressure elevation can be achieved by placing a flow resistance in the neck of the reservoir bag (15, 88). When such resistance is used, airway opening pressure should be monitored during the exhalation, that is, as shown in Figure 1. From the knowledge of the airway opening pressure and the resistance, air flow can be calculated. By ensuring a constant pressure during the exhalation, a constant flow will also be achieved.

Nose clips. Several groups routinely require patients to wear nose clips during the gas collection maneuver (15, 18, 46, 96), whereas other investigators do not (2, 16, 59). Unpublished data concerning a small population of 14 patients, some with asthma and some without, suggest that the wearing of nose clips has no effect on exhaled NO; mixed expired NO values were 6.2 ± 0.9 ppb with nose clips and 5.7 ± 0.9 ppb without nose clips. Given that there are no definitive data suggesting a requirement for nose clips, it seems reasonable to assume that for both fractionated and unfractionated collections, as long as measures are taken to ensure that (*1*) the soft palate is closed and gas from the nasopharynx is isolated from the collected sample and (*2*) the inspiration and expiration occur through the mouth, nose clips are not required.

Storage vessel. The reservoir used to collect the exhaled gas sample must be nonreactive and relatively impermeable to NO. Suitable materials include Tedlar and Mylar (15, 62, 88). It has been demonstrated that new Mylar balloons allow for sample stability for at least 48 h (15). Because no standardized vessels are available, the investigator must ensure that the reservoirs used are leak-free (both with regard to loss of sample to the atmosphere and to contamination by ambient NO), stable, and nonreactive. This can be accomplished by assaying several samples of varying NO concentration serially in the period of time and under ambient NO conditions appropriate for the experimental protocol employed. It should be noted that a given vessel may deteriorate over time; individual vessel integrity needs to be established at the time of its experimental use.

4. RECOMMENDATIONS FOR ONLINE AND OFFLINE MEASUREMENT OF EXHALED NITRIC OXIDE AND NASAL NITRIC OXIDE IN CHILDREN

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As with adults, there are several pediatric studies that show increases in exhaled NO in children with asthma compared with unaffected children, and a decrease in exhaled NO in children receiving inhaled corticosteroids (21–24, 38, 129–131). In addition, exhaled NO is generally lower than normal in children with cystic fibrosis (21, 38). The techniques for measurement used in these studies include (*1*) online measurement, which has proven difficult for some preadolescent subjects (87, 132); (*2*) vital capacity exhalation, which compares favorably with online single-breath exhalation (15, 22, 132, 133); and (*3*) analysis of online tidal breathing NO profiles against expiratory pressure (23, 134, 135).

Recommended Online Exhaled NO Measurement in Children

General aspects. The patient should remain comfortably seated, breathing room temperature air for 5 min before the test to acclimatize to laboratory conditions. The inspired gas should contain < 5 ppb NO (59).

Patients 12 yr old or older. NO exhaled by children ≥ 12 yr old should be measured by the same technique recommended for adults (*see* Section 2). If the patient is unable to perform the adult test, then testing should be performed as for children less than 12 yr old.

Patients less than 12 yr old. An expiratory flow rate of 50 ml/s, a dead space of ≤ 10 ml, and a 2-s plateau duration should be used for children less than 12 yr old (87, 136). Note that this differs from the adult protocol only in that the dead space and plateau duration are lower. The flow rate of 50 ml/s ensures an acceptable time to plateau and an acceptable rate of decline in lung volumes (87, 132), particularly for children with significant pathology who have vital capacities of less than 1 L (137). The expiratory pressure is maintained between 5 and 20 cm H_2O to ensure velum closure (59). Subjects inhale to TLC, and then exhale at a constant rate of 50 ml/s until at least a 2-s NO plateau has been achieved and exhalation has lasted for at least 4 s. Repeated exhalations are performed until three NO plateau values agree at the 10% level or two agree at the 5% level. There should be at least a 30-s interval between tests, to allow patients to rest. The mean NO value is then recorded. For children unable to sustain a steady expiration flow, forced vital capacity offline collection may be ideal (*see below*).

Recommended Offline Collection of Exhaled NO in Children

Offline NO collection has advantages in certain situations (*see* Section 3). Many studies of NO exhalation by children have

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been performed by offline methods (22, 138). The adult standard for offline NO collection (*see* Section 3), which has features that ensure nasal NO exclusion and constant flow exhalation, is recommended for children.

Children Unable to Cooperate

Children of any age may be unable to cooperate with the online and offline techniques, which require expiratory flow control. For these children the following two techniques may be employed.

Tidal breathing offline collection. The subject performs relaxed tidal breathing of NO-free air during nasal occlusion. The subject inspires orally via a one-way valve and expires against a resistance of at least 2 cm H_2O. Tidal expirate is collected in a light-impermeable Mylar bag or other suitable collection vessel. The mixed expired NO concentration is measured from the collection vessel after 2 min of tidal breathing.

Online tidal breathing method. Studies of online tidal breathing measurement against an expiratory pressure are highly repeatable, successfully exclude nasal air, and have shown robust differences between exhaled NO in asthmatic and normal subjects (23, 134, 135). The subject wears a nose clip to prevent nasal inhalation and breathes air passed through an NO scrubber and segregated from exhaled air by one-way valves. Nitric oxide levels are analyzed continuously during mouth breathing by a chemiluminescence analyzer, sampling at a constant flow. The exhaled air that is not withdrawn by sampling is discarded through a one-way valve to prevent contamination with ambient air. To keep the soft palate closed, an internal expiratory restrictor is used that creates a positive pressure of 3–4 cm H_2O at the mouthpiece. With a fast-reacting NO analyzer (response time < 500 ms), tidal NO profiles show a peak–trough–peak pattern or a peak pattern. Once the breathing pattern has stabilized, the exhaled NO portion is interpreted for each breath from the trough portion of the tracings in the former case or the peak portion in the latter case, assessed on multiple tidal breaths recorded over 1 min (83).

Exhaled Nitric Oxide Measurements in Ventilated Patients and Canopy Measurements

The state of the art in the areas of exhaled NO measurements in ventilated patients and canopy measurements is rapidly advancing but is not considered sufficient to provide detailed recommendations. However, it is agreed that (*1*) studies of V_{NO} in children should include information about the intake flow rate of the NO analyzer and the site of the sampling port; (*2*) studies in ventilated patients must be done without bias flow in the circuit; and (*3*) intrinsic losses of NO in the circuit between the patient and the sampling site must be measured and reported.

Nasal NO Measurement in Children

Nasal NO has been measured in children (21, 36, 37, 51, 55, 130, 138, 139) in the context of acute sinusitis, cystic fibrosis, and Kartagener syndrome. The use of audiovisual aids designed for children should facilitate the measurements.

Recommended method. The adult method is recommended for children who are able to cooperate (*see* Section 5), with a transnasal flow of 3 L/min and exclusion of lower respiratory tract air from the nasal cavity by closure of the velum (*see* Section 5) by any method shown to be reliable. Thus, Baraldi and coworkers have successfully used the nasal aspiration method during breathhold with closed glottis in children 5 yr of age and older (138, 139).

5. RECOMMENDATIONS FOR STANDARDIZED MEASUREMENT OF NASAL NITRIC OXIDE

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Evaluation and comparison of standardized methods for measurement of upper airway NO output are less developed, compared with measurements of lower airway NO output, but interest in this area is increasing. The supravelar airway can generate NO concentrations severalfold greater than in the lower respiratory tract, in the parts per million range (18, 45, 47), and the NO concentration is particularly high in the paranasal sinuses (47, 77). The nasal airway is a complex system of communicating cavities, that is, the nasal cavities, paranasal sinuses, middle ear, and nasopharynx. Each of these areas may contribute to nasal NO output. Measurements of nasal NO output or concentration cannot provide evidence as to the source of the gas (e.g., nasal cavity and/or paranasal sinuses) or the biochemical processes that generate the NO output (140). The nasal cavity has a unique vasculature that results in variation in nasal cavity volume, and alteration in nasal blood flow and/or volume could theoretically affect nasal NO production and absorption.

General Considerations

Measurement of nasal NO output requires generation of air flow through the nasal cavity (transnasal air flow). Flow through the nasal cavities in series can be achieved by aspirating or insufflating air via one naris while the velum is closed, so that air circulates from one naris to the other around the posterior nasal septum. Transnasal flow with the nasal cavities in parallel can be achieved by exhaling via one or both nasal cavities, by aspirating via the mouth with air entrained into both nares during breathholding, or by aspirating from one or both nares with the mouth open during breathholding. At present, little is known about effects of transnasal airflow direction on NO output. The transnasal flow in parallel mimicks natural nasal breathing. In subjects with unilateral or bilateral nasal obstruction, transnasal airflow may be decreased or absent, making measurement of nasal NO output more difficult or impossible.

With all methods, a constant transnasal flow rate produces a washout phase followed by the establishment of a steady NO plateau seen in the profile of NO versus time, analogous to that seen in the lower respiratory tract (Figure 6). The nasal NO concentration is inversely related to the transnasal airflow rate (82, 83, 141) (Figure 7). However, different flow rates may have different aerodynamic profiles, resulting in changes in the physics of airflow (e.g., laminar versus turbulent flow) and different pathways of flow through the nasal passages (142). The aerody-

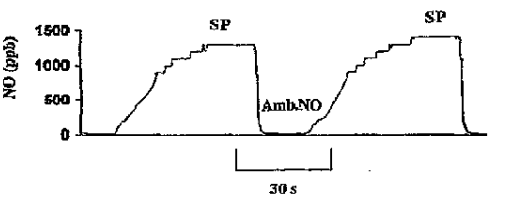

Figure 6. Two reproducible NO profiles versus time from a nasal NO measurement, using Method 1, showing a washout phase and a steady NO plateau (SP). In this case, the sampling line of the NO analyzer was used to generate the flow (200 ml/min). Amb. NO = ambient NO.

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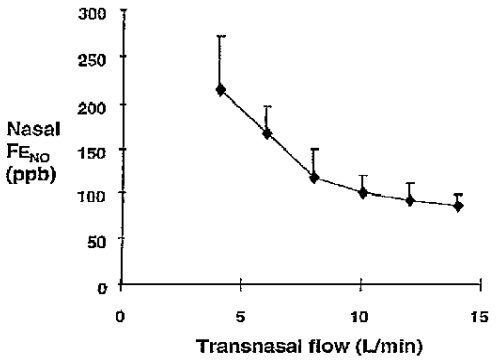

Figure 7. The transnasal flow dependence of nasal NO (83).

namics of this flow may affect nasal NO output (143, 144). For all of the preceding reasons, any standardized method should include rigorous control of the transnasal airflow rate.

Nasal NO Output

The product of transnasal flow rate ($\dot{V}$) and measured NO concentration allows calculation of NO output (V_{NO}). Present evidence suggests that V_{NO} is relatively constant over a range of transnasal flow rates between 1 and 5 L/min (82, 143–145). There is reasonable agreement, using different measurement techniques, that nasal NO output is in the range of 205–455 nl/min in healthy primates (45, 55, 141, 146). At transnasal flow rates < 0.3 L/min, NO may be taken up by nasal tissues, reducing the calculated V_{NO} (141). At higher flow rates, V_{NO} may increase progressively (145, 147).

The Importance of Velum Closure in Nasal NO Measurement

With transnasal airflow in series, velum closure is required to prevent loss of nasal NO via the posterior velopharyngeal aperture, or entry of lower respiratory air into the nasal cavity. Velum closure can be achieved in several ways:

1. Slowly exhaling orally against a resistance (59)
2. Pursed lips breathing via the mouth (148)
3. Breathholding with velum closed (46)
4. Voluntary elevation of the soft palate by a trained subject (145)

During the nasal NO test, measurement of nasal CO_2, which should remain low, can be used to verify velum closure.

Recommended Method for Measurement of Nasal NO

While several methods have been described for nasal NO measurement, the recommended method involves aspiration at constant flow from one naris with gas entrained via the other naris. This is currently the most prevalently used and best validated method (50, 53, 58, 83, 138, 139, 143, 147–150), and samples nasal NO in isolation from the lower respiratory tract. Velum closure is required to prevent leak of nasal NO via the posterior velopharyngeal aperture. Although several methods can be used to close the velum, slow oral exhalation against a resistance of at least 10 cm H_2O has been chosen as the preferred method (141), as this has been shown to close the velum reliably (59). A biofeedback display of airway pressure to the patient facilitates maintenance of a steady exhalation pressure within the desired range. Notwithstanding, any method that has been reliably demonstrated to close the velum is acceptable. The apparatus required for the recommended nasal measurement technique is shown in Figure 8.

Description of Method

Two nasal olives with a central lumen are securely placed in the nares, and used to aspirate air via one naris and entrain air via the other. These olives should be composed of a soft, nontraumatizing material, and of sufficient diameter and shape to occlude the naris. The seated subject inserts a mouthpiece, inhales to TLC, and exhales against expiratory resistance while targeting a mouth pressure of 10 cm H_2O to close the velum. While this exhalation is proceeding, air is aspirated at constant flow via one olive by a suction pump. A side port just distal to the aspirating olive samples gas for the NO analysis. An acceptable alternative to aspiration of air via a suction pump is insufflation of air from a constant flow, positive pressure source (e.g., medical-grade compressed air) into one nostril and sampling of nasal NO as air exits the other nostril (83). This insufflation method may be desirable when nasal cavity obstruction leads to dynamic alar collapse during the aspiration technique.

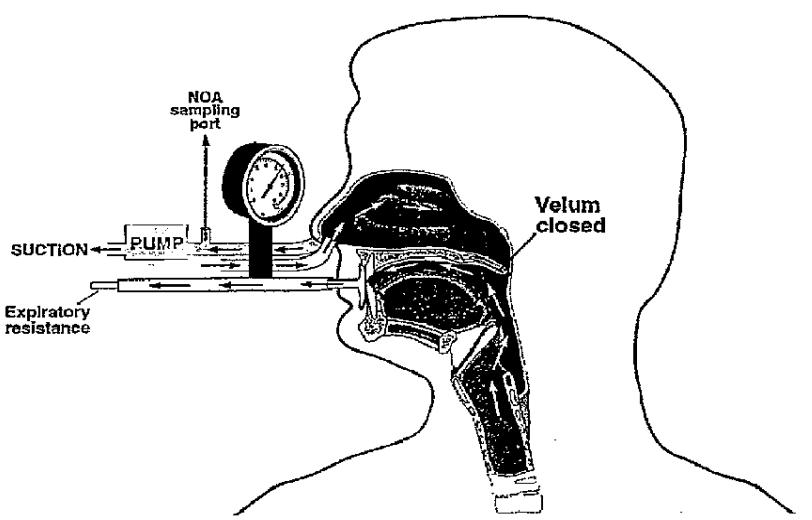

Figure 8. The transnasal airflow pattern employed in the recommended method for nasal NO measurement (*see text* for explanation). NOA = nitric oxide analyzer.

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However, insufflation of air under positive pressure may increase the likelihood of leakage of nasal air across the velum, and thus requires confirmation of velum closure (145).

Transnasal Airflow Rate

A target airflow rate of 3 L/min (50 ml/s) should be used in the measurement of nasal NO output, as this flow provides a steady plateau level of NO concentration in most patients within 20 s. This flow rate is also close to the physiologic range of ventilation flow through one side of the nasal cavity in a resting adult human and provides a turbulent flow pattern that facilitates ventilation of the nasal cavity (143). If a steady plateau of NO concentration is not achieved at this flow rate, other flow rates (in the range of 3–6 L/min) may be used to obtain a steady plateau NO concentration, as NO output is relatively stable in individual subjects over this flow range (82, 143–145). The precise flow used should be recorded with the NO measurement for each subject.

Factors Influencing Nasal NO Values

As with lower respiratory tract NO, the factors that specifically affect nasal NO are not well defined; the following discussion should serve to alert investigators to their possible influence on results.

Ambient air. Methods that use ambient air as the gas source for transnasal flow may introduce considerable NO concentrations (up to several hundred parts per billion) into the nasal cavity. It is conceivable that this extraneous NO may influence nasal physiology, but more importantly, it may reduce the gradient for NO diffusion from nasal epithelium to lumen. In an extreme situation, if ambient NO concentrations were greater than nasal mucosal wall concentrations, no net excretion of nasal NO would occur. For these reasons, it is preferable to use a clean air source. In any case, ambient NO should always be recorded at the time of each test and must be taken into account when assessing results.

Circadian change. As the presence or absence of a circadian effect on nasal NO has not been determined, it is reasonable to record the time and to attempt to measure nasal NO at the same time each day when performing serial measurements.

Posture. It would seem advisable to study patients in the seated position, which is the most convenient. In one study nasal NO was unchanged when the supine posture was assumed (151) although this increases nasal volume (152).

Age. Nasal NO does not appear to be age dependent beyond 11 yr of age, but in children ≤ 11 yr old it may affect NO output (47).

Sex. There are no data on effect of sex, menstrual cycle, or pregnancy on nasal NO output, but these characteristics should be noted in the record.

Body size/surface area. NO output, corrected for body surface area, is higher in children less than 11 yr old (144), but further studies are required in adults and children. In any case, height and weight should always be reported to allow calculations of NO output/body surface area ($\dot{V}_{NO}/m^2$).

Exercise. Nasal NO concentration falls during intense physical exercise (80, 82, 114). It is therefore prudent to refrain from exercise for 1 h before measurements are made.

Local Nasal Factors Affecting Nasal NO

Alterations in local nasal physiology could affect nasal NO, or may be mediated by nasal NO.

Nasal volume. Changes in nasal cavity volume could affect nasal NO by altering NO uptake into nasal blood, and by modulating the nasal epithelial surface area. Also, the communication of the nasal cavity with the communicating sinuses, which produce NO, could be altered. Evidence concerning the influence of nasal volume on nasal NO is contradictory at present. Nasal NO output was not volume dependent, provided a true steady state plateau was achieved, in one study (151) but has been reported to be volume dependent at low transnasal flow rates in another (153), possibly owing to changes in nasal aerodynamics (143).

Nasal aerodynamics. The physics of airflow through the nasal cavity could alter the sampling of nasal NO. At low flows, laminar flow may predominate, and certain areas of the cavity may contribute less NO to the sample. Also at low flows, the pressure fluxes in the nasal cavity will be less than at high flows, possibly reducing the efflux of gas from the paranasal sinuses. Variations in nasal aerodynamics may explain some of the flow dependency of nasal NO output (143).

Medications and Nasal NO

Medications have been shown to affect NO and should be recorded. Those reported to have an effect on nasal NO include nasal decongestants (44, 142), which decrease nasal NO output by about 15% (151, 153). The routine use of decongestants to facilitate nasal NO measurement itself requires further study. Nasal steroids have been reported to have no effect in normal subjects in one study (55), but to reduce nasal NO output after 2 wk of therapy in normal subjects (78) and asthmatics (23) in other reports. Antibiotic therapy had no effect on nasal NO in normal subjects in one study (154) but nasal NO rose after treatment of sinusitis in another (138). Vasodilators (e.g., papaverine) increased nasal NO output in one report (155) whereas histamine had no effect in another study (153). Saline does not appear to affect nasal NO output (151) but lidocaine may have a differential effect on nasal and sinus NO output (140).

Nitric oxide synthase inhibitors. L-NAME administered by nasal spray has been reported to have no effect in some studies (47, 77, 153), but also to decrease NO output (155, 156).

L-Arginine. L-Arginine is the substrate for NO synthesis. Systemic administration increased nasal NO output by 35% in one study (121) but had no effect when applied by nasal spray in normal patients (153).

Smoking

A small decrease in nasal NO has been observed in smokers (145).

6. EQUIPMENT RECOMMENDATIONS FOR MEASUREMENT OF EXHALED NO

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Current NO analyzers employ the principle of chemiluminesence to measure NO. However, NO measurement based on alternative technologies may be developed in future. Equipment needs will vary according to the applications and test procedures. The following recommendations therefore refer to the proposed application.

Online Analysis of Exhaled and Nasal NO in Adults and Children

Table 1 displays the current minimum specifications required for accurate online measurement of exhaled NO and nasal NO. Exhalation flow rates for adults and children (0.05 L/s) are measured online at 37° C, 760 mm Hg saturated (BTPS), in keeping with other pulmonary function measurements.

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Breath-by-Breath Analysis

Breath-by-breath analysis may be necessary for young children and in ventilated subjects although the latter are not covered in this document. Each investigator will need to select an analyzer that is adequate in terms of response time and sampling rate for the particular frequency of the breaths or mechanical ventilation.

Offline Analysis

For offline analysis, the preceding specifications for online analysis apply with regard to sensitivity, accuracy, and range. The analyzer response time and tubing/setup lag time are not relevant. The specific requirements include the ability to display a steady NO plateau of at least 3 s by adjustment of the sample inlet flow, thus permitting a reliable signal. This is especially important with small volume samples, for example, from young children.

Material Requirements

Acceptable materials for tubing, connectors, and so on, include Teflon, stainless steel, siliconized materials, and Teflon-coated materials (for greater flexibility). Latex-related materials are not acceptable, owing to interference, including reaction with NO.

Calibration Requirements and Procedures

Zero NO gas. A reliable zero NO gas is essential for NO measurements. It is recommended that, rather than relying on medical-grade air, NO scrubbers ($KMnO_4$ and/or charcoal) be used to generate zero NO gas. A good zero NO gas can be prepared by passing ambient air through an ozone generator, which converts any NO to NO_2 before entry into the analyzer (NO knockout method).

Upper point calibration. Upper point calibration requires specially prepared NO calibration gases, most commonly in nitrogen. Commonly available concentrations range from 2 to 100 ppm, although levels as low as 100 ppb are available. Although standard gases may be supplied at the ± 2 or ± 5% guaranteed accuracy level, the former is highly recommended to optimize accuracy and reproducibility of analysis of unknown samples. Gases should be guaranteed to be stable for > 6 mo. While calibrations in the parts per million range performed for measurements made in the parts per billion range are suboptimal, stable parts per billion calibration gases are not widely available. Commercially available high-accuracy gas dilution systems permit generation of parts per billion gases from parts per million standards.

Calibration range. For each analyzer, it is suggested that investigators perform an initial linear validation, using at least a three-point calibration (zero and two higher NO concentrations) in the expected range of sample values: 100- to 1,000-ppb range for expired samples and 0.4- to 50-ppm range for nasal samples.

Frequency of calibration. Daily calibrations should be performed, using the zero NO gas and one other concentration in the expected range of sample values.

Factors affecting calibration. The gas sample flow rate to the analyzer should be checked at regular intervals (e.g., weekly), as the chemiluminesence signal is sensitive to fluctuations in flow and reaction chamber pressure. Calibration and NO sampling should always be performed at the same sample flow rate. Calibration should be repeated if ambient laboratory conditions change.

TABLE 1

MINIMUM SPECIFICATIONS FOR ONLINE MEASUREMENT OF EXHALED AND NASAL NITRIC OXIDE

Parameter	Value
Sensitivity	1 ppb (noise, < 0.5 ppb)
Signal/noise ratio	> 2
Accuracy	Exhaled NO: Better than 1 pbb Nasal NO: Better than 0.1 ppm
Range	Exhaled NO: 1–500 ppb Nasal NO: 0.1–50 ppm
Response time*	< 500 ms
Lag time*	To be measured and reported by the investigator
Drift	Less than 1% of full scale per 24 h
Reproducibility	Exhaled NO: Better than 1 pbb Nasal NO: Better than 0.1 ppm
Flowthrough sensor	To be measured by manufacturer and reported in publications

* Response time is defined as the delay from introduction of a square-wave signal and achievement of 90% of the maximum signal, inclusive of electronic delays and physical delays because of sample introduction, but not including tubing length. Lag time includes delays due to transit time through sample tubing in a particular application.

Influence of Extraneous Factors on NO Analysis

Ambient conditions. The instruments are fairly sensitive to ambient conditions, including exposure to sunlight, temperature, humidity, and so on. Due diligence should be taken to confirm stable ambient conditions, failing which the zero point should be rechecked before each sample is taken. For example, ambient temperature should not vary by more than 1° C from the time of calibration.

Humidity. With regard to sample humidity, drying the sample by passing it through a filter containing crystals (e.g., Drierite) may absorb NO and is therefore not recommended. The possible error in NO measurement due to humidity should be addressed by each manufacturer. One approach is to use a Nafion tube in the sample line, which equilibrates the sample with ambient humidity. In any case, steps should be taken to ensure that calibration gases (dry, ambient temperature) and samples (saturated, 37° C) are at the same humidity and temperature.

Interfering substances. Interfering substances include volatile anesthetic gases, which may be hazardous to the measurement system with regard to chemical reactions, oxidation of analyzer and tubing materials, and so on. Tolerances to quenching by CO_2 and water vapor, which affect NO analysis (157), should be < 1% NO per 1% level of interfering substance. Alcohol-containing disinfectants interfere with NO analysis (158).

Ancillary Features and Equipment

NO analysis specifications as detailed here are essential to the reliable measurement and reporting of exhaled NO data. However, some additional features will facilitate NO measurements according to the recommendations for a standardized technique in this statement. The following list of features would be part of an integrated NO measurement, analysis, and data-handling system:

Output: Provision of both analog and digital output, RAM storage card

Data collection capability: The following features may be helpful:

- Transmission of collected NO output data (NO, pressure, flow) to computer or monitor for real-time display
- Automatic sensing and indication of quality of exhalation, achievement of valid NO plateau according to that

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defined in this statement (*see* Section 2), allowing termination of exhalation
- Data storage
- Data analysis software allowing manipulation and display of results, etc.

Biofeedback of exhalation parameters: For adult and pediatric measurement of exhaled NO and nasal NO, biofeedback of exhalation parameters may be essential for those systems that generate constant flow in this manner

Sample flow rate: Automatic monitoring and display of NO analyzer sample flow rate, e.g., by including a rotameter

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References

1. Shelhamer, J. H., S. J. Levine, T. Wu, D. B. Jacoby, M. A. Kaliner, and S. I. Rennard. 1995. NIH conference: airway inflammation. *Ann. Intern. Med.* 123:288–304.
2. Gustaffson, L. E., A. M. Leone, M. G. Persson, N. P. Wiklund, and S. Moncada. 1991. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem. Biophys. Res. Commun.* 181(2):852–857.
3. Zayasu, K., K. Sekizawa, S. Okinaga, M. Yamaya, T. Ohrui, and H. Sasaki. 1997. Increased carbon monoxide in exhaled air of asthmatic patients. *Am. J. Respir. Crit. Care Med.* 156:1140–1143.
4. Horvath, I., L. E. Donnelly, A. Kiss, P. Paredi, S. A. Kharitonov, and P. J. Barnes. 1998. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. *Thorax* 53: 668–672.
5. Yamaya, M., K. Sekizawa, S. Ishizuka, M. Monma, K. Mizuta, and H. Sasaki. 1998. Increased carbon monoxide in exhaled air of subjects with upper respiratory tract infections. *Am. J. Respir. Crit. Care Med.* 158:311–314.
6. Ortolani, O., A. Conti, M. Biasiucci, G. Crescenzi, and R. Imperatore. 1986. Free radical lipid peroxidation through expired ethane and pentane: an improved method. *Boll. Soc. Ital. Biol. Sper.* 62:383–389.
7. Kneepkens, C. M., C. Ferreira, G. Lepage, and C. C. Roy. 1992. The hydrocarbon breath test in the study of lipid peroxidation: principles and practice. *Clin. Invest. Med.* 15:163–186.
8. Seabra, L., J. M. Braganza, and M. F. Jones. 1991. A system for the quantitative determination of hydrocarbons in human breath. *J. Pharm. Biomed. Anal.* 9:693–697.
9. Nowak, D., A. Antczak, M. Krol, T. Pietras, B. Shariati, P. Bialasiewicz, K. Jeczkowski, and P. Kula. 1996. Increased content of hydrogen peroxide in the expired breath of cigarette smokers. *Eur. Respir. J.* 9:652–657.
10. Dekhuijzen, P. N., K. K. Aben, I. Dekker, L. P. Aarts, P. L. Wielders, C. L. van Herwaarden, and A. Bast. 1996. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 154:813–816.
11. Dohlman, A. W., H. R. Black, and J. A. Royall. 1993. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. *Am. Rev. Respir. Dis.* 148:955–960.
12. Kietzmann, D., R. Kahl, M. Muller, H. Burchardi, and D. Kettler. 1993. Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS. *Intens. Care Med.* 19:78–81.
13. Gaston, B. M., J. M. Drazen, C. B. E. Chee, M. E. B. Wohl, and J. S. Stamler. 1994. *In* Biology of Nitric Oxide: Clinical and Physiological Implications. Portland Press, London. 497–500.
14. Kharitonov, S. A., D. Yates, D. R. Springall, L. Buttery, J. Polak, R. A. Robbins, and P. J. Barnes. 1995. Exhaled nitric oxide is increased in asthma. *Chest* 107:156S–157S.
15. Massaro, A. F., B. Gaston, D. Kita, C. Fanta, J. S. Stamler, and J. M. Drazen. 1995. Expired nitric oxide levels during treatment of acute asthma. *Am. J. Respir. Crit. Care Med.* 152:800–803.
16. Kharitonov, S. A., D. Yates, R. A. Robbins, R. Logan-Sinclair, E. A. Shinebourne, and P. J. Barnes. 1994. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 343:133–135.
17. Persson, M. G., O. Zetterstrom, V. Agrenius, E. Ihre, and L. E. Gustafsson. 1994. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 343:146–147.
18. Alving, K., E. Weitzberg, and J. M. Lundberg. 1993. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur. Respir. J.* 6:1368–1370.
19. Yates, D. H., S. A. Kharitonov, R. A. Robbins, P. S. Thomas, and P. J. Barnes. 1995. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 152:892–896.
20. Silkoff, P. E., P. McClean, A. S. Slutsky, M. Caramori, K. R. Chapman, C. Gutierrez, and N. Zamel. 1998. Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma. *J. Asthma* 35(6):473–479.
21. Lundberg, J. O., S. L. Nordvall, E. Weitzberg, H. Kollberg, and K. Alving. 1996. Exhaled nitric oxide in paediatric asthma and cystic fibrosis. *Arch. Dis. Child.* 75:323–326.
22. Nelson, B. V., S. Sears, J. Woods, C. Y. Ling, J. Hunt, L. M. Clapper, and B. Gaston. 1997. Expired nitric oxide as a marker for childhood asthma. *J. Pediatr.* 130:423–427.
23. Baraldi, E., N. M. Azzolin, S. Zanconato, C. Dario, and F. Zacchello. 1997. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. *J. Pediatr.* 131:381–385.
24. Byrnes, C. A., S. Dinarevic, E. A. Shinebourne, P. J. Barnes, and A. Bush. 1997. Exhaled nitric oxide measurements in normal and asthmatic children. *Pediatr. Pulmonol.* 24:312–318.
25. Maziak, W., S. Loukides, S. Culpitt, P. Sullivan, S. A. Kharitonov, and P. J. Barnes. 1998. Exhaled nitric oxide in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 157:998–1002.
26. Kharitonov, S. A., A. U. Wells, B. J. O'Connor, P. J. Cole, D. M. Hansell, R. B. Logan-Sinclair, and P. J. Barnes. 1995. Elevated levels of exhaled nitric oxide in bronchiectasis. *Am. J. Respir. Crit. Care Med.* 151:1889–1893.
27. Ho, L. P., J. A. Innes, and A. P. Greening. 1998. Exhaled nitric oxide is not elevated in the inflammatory airways diseases of cystic fibrosis and bronchiectasis. *Eur. Respir. J.* 12:1290–1294.
28. Kharitonov, S. A., D. Yates, and P. J. Barnes. 1995. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur. Respir. J.* 8:295–297.
29. de Gouw, H. W., K. Grunberg, R. Schot, A. C. Kroes, E. C. Dick, and P. J. Sterk. 1998. Relationship between exhaled nitric oxide and air-

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way hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur. Respir. J.* 11:126–132.
30. Rolla, G., L. Brussino, M. T. Bertero, P. Colagrande, M. Converso, C. Bucca, S. Polizzi, and F. Caligaris-Cappio. 1997. Increased nitric oxide in exhaled air of patients with systemic lupus erythematosus. *J. Rheumatol.* 24:1066–1071.
31. Soderman, C., A. Leone, V. Furst, and M.G. Persson. 1997. Endogenous nitric oxide in exhaled air from patients with liver cirrhosis. *Scand. J. Gastroenterol.* 32:591–597.
32. Sogni, P., P. Garnier, A. Gadano, R. Moreau, J. Dall'Ava-Santucci, A. T. Dinh-Xuan, and D. Lebrec. 1995. Endogenous pulmonary nitric oxide production measured from exhaled air is increased in patients with severe cirrhosis. *J. Hepatol.* 23:471–473.
33. Cremona, G., T. W. Higenbottam, V. Mayoral, G. Alexander, E. Demoncheaux, C. Borland, P. Roe, and G. J. Jones. 1995. Elevated exhaled nitric oxide in patients with hepatopulmonary syndrome. *Eur. Respir. J.* 8:1883–1885.
34. Rolla, G., L. Brussino, P. Colagrande, L. Dutto, S. Polizzi, E. Scappaticci, S. Bergerone, M. Morello, A. Marzano, G. Martinasso, M. Salizzoni, and C. Bucca. 1997. Exhaled nitric oxide and oxygenation abnormalities in hepatic cirrhosis. *Hepatology* 26:842–847.
35. Silkoff, P. E., M. Caramori, L. Tremblay, P. McClean, C. Chaparro, S. Kesten, M. Slutsky, A. S. Hutcheon, N. Zamel, and S. Keshavjee. 1998. Exhaled nitric oxide in human lung transplantation: a noninvasive marker of acute rejection. *Am. J. Respir. Crit. Care Med.* 157:1822–1828.
36. Dotsch, J., S. Demirakca, H. G. Terbrack, G. Huls, W. Rascher, and P. G. Kuhl. 1996. Airway nitric oxide in asthmatic children and patients with cystic fibrosis. *Eur. Respir. J.* 9:2537–2540.
37. Balfour-Lynn, I. M., A. Laverty, and R. Dinwiddie. 1996. Reduced upper airway nitric oxide in cystic fibrosis. *Arch. Dis. Child.* 75:319–322.
38. Grasemann, H., E. Michler, M. Wallot, and F. Ratjen. 1997. Decreased concentration of exhaled nitric oxide (NO) in patients with cystic fibrosis. *Pediatr. Pulmonol.* 24:173–177.
39. Grasemann, H., I. Ioannidis, R.P. Tomkiewicz, H. de Groot, B. K. Rubin, and F. Ratjen. 1998. Nitric oxide metabolites in cystic fibrosis lung disease. *Arch. Dis. Child.* 78:49–53.
40. Loveless, M. O., C. R. Phillips, G. D. Giraud, and W. E. Holden. 1997. Decreased exhaled nitric oxide in subjects with HIV infection. *Thorax* 52:185–186.
41. Riley, M. S., J. Porszasz, J. Miranda, M. P. Engelen, B. Brundage, and K. Wasserman. 1997. Exhaled nitric oxide during exercise in primary pulmonary hypertension and pulmonary fibrosis. *Chest* 111:44–50.
42. Cremona, G., T. Higenbottam, C. Borland, and B. Mist. 1994. Mixed expired nitric oxide in primary pulmonary hypertension in relation to lung diffusion capacity. *Q. J. Med.* 87:547–551.
43. Jatakanon, A., S. Lim, S. A. Kharitonov, K. F. Chung, and P. J. Barnes. 1998. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 53:91–95.
44. Dupont, L. J., F. Rochette, M. G. Demedts, and G. M. Verleden. 1998. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naive patients with mild asthma. *Am. J. Respir. Crit. Care Med.* 157:894–898.
45. Gerlach, H., R. Rossaint, D. Pappert, M. Knorr, and K. J. Falke. 1994. Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx [see comments]. *Lancet* 343:518–519.
46. Kimberly, B., B. Nejadnik, G. D. Giraud, and W. E. Holden. 1996. Nasal contribution to exhaled nitric oxide at rest and during breath-holding in humans. *Am. J. Respir. Crit. Care Med.* 153:829–836.
47. Lundberg, J. O., T. Farkas-Szallasi, E. Weitzberg, J. Rinder, J. Lidholm, A. Anggaard, T. Hokfelt, J. M. Lundberg, and K. Alving. 1995. High nitric oxide production in human paranasal sinuses. *Nature Med.* 1:370–373.
48. Xia, Y., and J. L. Zweier. 1997. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 94:6954–6958.
49. Jain, B., I. Rubinstein, R. A. Robbins, K. L. Leise, and J. H. Sisson. 1993. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem. Biophys. Res. Commun.* 191:83–88.
50. Kharitonov, S. A., K. Rajakulasingam, B. O'Connor, S. R. Durham, and P. J. Barnes. 1997. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J. Allergy Clin. Immunol.* 99:58–64.
51. Lundberg, J. O. 1996. Airborne nitric oxide: inflammatory marker and aerocrine messenger in man. *Acta Physiol. Scand. Suppl.* 633:1–27.
52. Martin, U., K. Bryden, M. Devoy, and P. Howarth. 1996. Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis. *J. Allergy Clin. Immunol.* 97:768–772.
53. Arnal, J. F., A. Didier, J. Rami, C. M'Rini, J. P. Charlet, E. Serrano, and J. P. Besombes. 1997. Nasal nitric oxide is increased in allergic rhinitis. *Clin. Exp. Allergy* 27:358–362.
54. Garrelds, I. M., J. G. van Amsterdam, C. de Graaf-in't Veld, R. Gerth van Wijk, and F. J. Zijlstra. 1995. Nitric oxide metabolites in nasal lavage fluid of patients with house dust mite allergy. *Thorax* 50:275–279.
55. Lundberg, J. O., E. Weitzberg, S. L. Nordvall, R. Kuylenstierna, J. M. Lundberg, and K. Alving. 1994. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. *Eur. Respir. J.* 7:1501–1504.
56. Lindberg, S., A. Cervin, and T. Runer. 1997. Low levels of nasal nitric oxide (NO) correlate to impaired mucociliary function in the upper airways. *Acta. Otolaryngol. (Stockholm)* 117:728–734.
57. Kharitonov, S. A., and P. J. Barnes. 1997. Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath holding. *Thorax* 52:540–544.
58. Kharitonov, S., K. Alving, and P. J. Barnes. 1997. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur. Respir. J.* 10:1683–1693.
59. Silkoff, P. E., P. A. McClean, A. S. Slutsky, H. G. Furlott, E. Hoffstein, S. Wakita, K .R. Chapman, J. P. Szalai, and N. Zamel. 1997. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am. J. Respir. Crit. Care Med.* 155:260–267.
60. Tsujino, I., K. Miyamoto, M. Nishimura, H. Shinano, H. Makita, S. Saito, T. Nakano, and Y. Kawakami. 1996. Production of nitric oxide (NO) in intrathoracic airways of normal humans. *Am. J. Respir. Crit. Care Med.* 154:1370–1374.
61. Sato, K., T. Sakamaki, H. Sumino, H. Sakamoto, J. Hoshino, H. Masuda, Y. Sawada, M. Mochida, Y. Ohyama, T. Kurashina, T. Nakamura, and Z. Ono. 1996. Rate of nitric oxide release in the lung and factors influencing the concentration of exhaled nitric oxide. *Am. J. Physiol.* 270:L914–L920.
62. Robbins, R. A., A. A. Floreani, S. G. Von Essen, J. H. Sisson, G. E. Hill, I. Rubinstein, and R. G. Townley. 1996. Measurement of exhaled nitric oxide by three different techniques. *Am. J. Respir. Crit. Care Med.* 153:1631–1635.
63. Morris, N. H., S. Carroll, K. H. Nicolaides, P. J. Steer, and J. B. Warren. 1995. Exhaled nitric oxide concentration and amniotic fluid nitrite concentration during pregnancy. *Eur. J. Clin. Invest.* 25:138–141.
64. Mehta, S., D. J. Stewart, and R. D. Levy. 1996. The hypotensive effect of L-arginine is associated with increased expired nitric oxide in humans. *Chest* 109:1550–1555.
65. Kharitonov, S. A., K. F. Chung, D. Evans, B. J. O'Connor, and P. J. Barnes. 1996. Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 153:1773–1780.
66. Massaro, A. F., S. Mehta, C.M. Lilly, L. Kobzik, J. J. Reilly, and J. M. Drazen. 1996. Elevated nitric oxide concentrations in isolated lower airway gas of asthmatic subjects. *Am. J. Respir. Crit. Care Med.* 153: 1510–1514.
67. Silkoff, P. E., P. McClean, M. Caramori, A. S. Slutsky, and N. Zamel. 1998. A significant proportion of exhaled nitric oxide arises in large airways in normal subjects. *Respir. Physiol.* 113(1):33–38.
68. Dweik, R. A., D. Laskowski, H. M. Abu-Soud, F. Kaneko, R. Hutte, D. J. Stuehr, and S. C. Erzurum. 1998. Nitric oxide synthesis in the lung. Regulation by oxygen through a kinetic mechanism. *J. Clin. Invest.* 101:660–666.
69. Silkoff, P. E., J. T. Silvester, N. Zamel, and S. Permutt. 1999. The exhaled nitric oxide system in the human airway; the relationship to pulmonary function and bronchial reactivity in asthma. *Am. J. Respir. Crit. Care Med.* (In press)
70. Tsoukias, N. M., Z. Tannous, A. F. Wilson, and S. C. George. 1998. Single-exhalation profiles of NO and CO_2 in humans: effect of dynamically changing flow rate. *J. Appl. Physiol.* 85(2):642–652.
71. Tsoukias, N. M., and S. C. George. 1998. A two compartment model of pulmonary nitric oxide exchange dynamics. *J. Appl. Physiol.* 85(2):653–666.
72. Byrnes, C. A., S. Dinarevic, C. Busst, A. Bush, and E. A. Shinebourne. 1997. Is nitric oxide in exhaled air produced at airway or alveolar level? *Eur. Respir. J.* 10:1021–1025.
73. Jorres, R. A., H. Sonnemann, J. Lohmann, and H. Magnussen. 1998. Determination of bronchial production characteristics of exhaled nitric oxide (NO) in humans (abstract). *Am. J. Respir. Crit. Care Med.* 157(3):A612.

PM3006723804

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74. Suzuki, H., and J. A. Krasney. 1997. Nitric oxide in single-breath exhalation in humans. *Jpn. J. Physiol.* 47:335–339.
75. Lundberg, J. O., E. Weitzberg, J. M. Lundberg, and K. Alving. 1994. Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* 35:1543–1546.
76. Schedin, U., C. Frostell, M. G. Persson, J. Jakobsson, G. Andersson, and L. E. Gustafsson. 1995. Contribution from upper and lower airways to exhaled endogenous nitric oxide in humans. *Acta Anaesthesiol. Scand.* 39:327–332.
77. Lundberg, J. O., J. Rinder, E. Weitzberg, J. M. Lundberg, and K. Alving. 1994. Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. *Acta Physiol. Scand.* 152:431–432.
78. Dillon, W. C., V. Hampl, P. J. Shultz, J. B. Rubins, and S. L. Archer. 1996. Origins of breath nitric oxide in humans. *Chest* 110:930–938.
79. Chatkin, J., W. Qian, P. McClean, N. Zamel, J. Haight, and P. E. Silkoff. 1999. Nitric oxide accumulation in the non-ventilated nasal cavity. *Arch. Otolaryngol.–Head Neck Surg.* 125(6):682–685.
80. Phillips, C. R., G. D. Giraud, and W. E. Holden. 1996. Exhaled nitric oxide during exercise: site of release and modulation by ventilation and blood flow. *J. Appl. Physiol.* 80:1865–1871.
81. Hogman, M., S. Stromberg, U. Schedin, C. Frostell, G. Hedenstierna, and L. E. Gustaffson. 1997. Nitric oxide from the human respiratory tract efficiently quantified by standardised single breath measurements. *Acta Physiol. Scand.* 159:345–346.
82. Imada, M., J. Iwamoto, S. Nonaka, Y. Kobayashi, and T. Unno. 1996. Measurement of nitric oxide in human nasal airway. *Eur. Respir. J.* 9:556–559.
83. Silkoff, P. E., J. Chatkin, W. Qian, S. Chakravorty, C. Gutierrez, H. Furlott, P. McClean, S. Rai, N. Zamel, and J. Haight. 1999. Nasal nitric oxide: a comparison of measurement techniques. *Am. J. Rhinol.* 13:169–178.
84. Persson, M. G., N. P. Wiklund, and L. E. Gustafsson. 1993. Endogenous nitric oxide in single exhalations and the change during exercise. *Am. Rev. Respir. Dis.* 148:1210–1214.
85. Jilma, B., J. Kastner, C. Mensik, B. Vondrovec, J. Hildebrandt, K. Krejcy, O. F. Wagner, and H. G. Eichler. 1996. Sex differences in concentrations of exhaled nitric oxide and plasma nitrate. *Life Sci.* 58:469–476.
86. Morris, N. H., S. R. Sooranna, P. J. Steer, and J. B. Warren. 1996. The effect of the menstrual cycle on exhaled nitric oxide and urinary nitrate concentration. *Eur. J. Clin. Invest.* 26:481–484.
87. Franklin, P. J., R. Taplin, and S. M. Stick. 1999. A community study of exhaled nitric oxide in healthy children. *Am. J. Respir. Crit. Care Med.* 159:69–73.
88. Deykin, A., O. Halpern, A. F. Massaro, J. M. Drazen, and E. Israel. 1998. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. *Am. J. Respir. Crit. Care Med.* 157:769–775.
89. Silkoff, P. E., J. Chatkin, P. McClean, K. R. Chapman, S. Rai, A. S. Slutsky, N. Zamel, and K. R. Chapman. 1999. Exhaled nitric oxide after beta-2 agonist inhalation and spirometry in bronchial asthma. *Am. J. Respir. Crit. Care Med.* 159:940–944.
90. de Gouw, H. W. F. M., J. Hendriks, A. M. Woltman, I. M. Twiss, and P. J. Sterk. 1998. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. *Am. J. Respir. Crit. Care Med.* 158:315–319.
91. Yates, D. H., S. A. Kharitonov, and P. J. Barnes. 1997. Effect of short- and long-acting inhaled beta2-agonists on exhaled nitric oxide in asthmatic patients. *Eur. Respir. J.* 10:1483–1488.
92. Silkoff, P. E., S. Wakita, J. Chatkin, K. Ansarin, C. Gutierrez, M. Caramori, P. McClean, A. S. Slutsky, N. Zamel, and K. R. Chapman. 1999. Exhaled nitric oxide after beta2-agonist inhalation and spirometry in asthma. *Am. J. Respir. Crit. Care Med.* 159:940–944.
93. Zetterquist, W., C. Pedroletti, J. O. Lundberg, and K. Alving. 1999. Salivary contribution to exhaled nitric oxide. *Eur. Respir. J.* 13:327–333.
94. Byrnes, C. A., S. Dinarevic, C. A. Busst, E. A. Shinebourne, and A. Bush. 1997. Effect of measurement conditions on measured levels of peak exhaled nitric oxide. *Thorax* 52:697–701.
95. Yates, D. H., S. A. Kharitonov, R. A. Robbins, P. S. Thomas, and P. J. Barnes. 1996. The effect of alcohol ingestion on exhaled nitric oxide. *Eur. Respir. J.* 9:1130–1133.
96. Persson, M. G., B. Cederqvist, C. U. Wiklund, and L. E. Gustafsson. 1994. Ethanol causes decrements in airway excretion of endogenous nitric oxide in humans. *Eur. J. Pharmacol.* 270:273–278.
97. Georges, G., B. Bucher-Bartelson, R. J. Martin, and P. E. Silkoff. 1999. Circadian variation in exhaled nitric oxide in nocturnal asthma. *J. Asthma* 36:467–473.
98. ten Hacken, N. H., H. van der Vaart, T. W. van der Mark, G. H. Koeter, and D. S. Postma. 1998. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. *Am. J. Respir. Crit. Care Med.* 158(3):902–907.
99. Robbins, R. A., T. Millatmal, K. Lassi, S. Rennard, and D. Daughton. 1997. Smoking cessation is associated with an increase in exhaled nitric oxide. *Chest* 112:313–318.
100. Kharitonov, S. A., R. A. Robbins, D. Yates, V. Keatings, and P. J. Barnes. 1995. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 152:609–612.
101. Schilling, J., P. Holzer, M. Guggenbach, D. Gyurech, K. Marathia, and S. Geroulanos. 1994. Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives. *Eur. Respir. J.* 7:467–471.
102. Pogliaghi, S., J. A. Krasney, and D. R. Pendergast. 1997. Effect of gravity on lung exhaled nitric oxide at rest and during exercise. *Respir. Physiol.* 107:157–164.
103. Schmetterer, L., K. Strenn, J. Kastner, H. G. Eichler, and M. Wolzt. 1997. Exhaled NO during graded changes in inhaled oxygen in man. *Thorax* 52:736–738.
104. Stromberg, S., P. A. Lonnqvist, M. G. Persson, and L. E. Gustafsson. 1997. Lung distension and carbon dioxide affect pulmonary nitric oxide formation in the anaesthetized rabbit. *Acta Physiol. Scand.* 159:59–67.
105. Carlin, R. E., L. Ferrario, J. T. Boyd, E. M. Camporesi, D. J. McGraw, and T. S. Hakim. 1997. Determinants of nitric oxide in exhaled gas in the isolated rabbit lung. *Am. J. Respir. Crit. Care Med.* 155:922–927.
106. Persson, M. G., P. A. Lonnqvist, and L. E. Gustafsson. 1995. Positive end-expiratory pressure ventilation elicits increases in endogenously formed nitric oxide as detected in air exhaled by rabbits. *Anesthesiology* 82:969–974.
107. Chirpaz-Oddou, M. F., A. Favre-Juvin, P. Flore, J. Eterradossi, M. Delaire, F. Grimbert, and A. Therminarias. 1997. Nitric oxide response in exhaled air during an incremental exhaustive exercise. *J. Appl. Physiol.* 82:1311–1318.
108. Massaro, A. F., and J. M. Drazen. 1996. Exhaled nitric oxide during exercise: site of release and modulation by ventilation and blood flow [Editorial]. *J. Appl. Physiol.* 80:1863–1864.
109. Bauer, J. A., J. A. Wald, S. Doran, and D. Soda. 1994. Endogenous nitric oxide in expired air: effects of acute exercise in humans. *Life Sci.* 55:1903–1909.
110. Iwamoto, J., D. R. Pendergast, H. Suzuki, and J. A. Krasney. 1994. Effect of graded exercise on nitric oxide in expired air in humans. *Respir. Physiol.* 97:333–345.
111. Maroun, M. J., S. Mehta, R. Turcotte, M. G. Cosio, and S. N. Hussain. 1995. Effects of physical conditioning on endogenous nitric oxide output during exercise. *J. Appl. Physiol.* 79:1219–1225.
112. Trolin, G., T. Anden, and G. Hedenstierna. 1994. Nitric oxide (NO) in expired air at rest and during exercise. *Acta Physiol. Scand.* 151:159–163.
113. Yasuda, Y., T. Itoh, M. Miyamura, and H. Nishino. 1997. Comparison of exhaled nitric oxide and cardiorespiratory indices between nasal and oral breathing during submaximal exercise in humans. *Jpn. J. Physiol.* 47:465–470.
114. Lundberg, J. O., J. Rinder, F. Weitzberg, K. Alving, and J. M. Lundberg. 1997. Heavy physical exercise decreases nitric oxide levels in the nasal airways in humans. *Acta Physiol. Scand.* 159:51–57.
115. Kharitonov, S. A., D. H. Yates, K. F. Chung, and P. J. Barnes. 1996. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. *Eur. Respir. J.* 9:196–201.
116. Kharitonov, S. A., D. H. Yates, and P. J. Barnes. 1996. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am. J. Respir. Crit. Care Med.* 153:454–457.
117. Kharitonov, S. A., *et al.* 1996. Reduction in exhaled nitric oxide after a single dose of nebulised bedenoside in patients with asthma (abstract). *Am. J. Respir. Crit. Care Med.* 153:A799–A790.
118. Yates, D. H., S. A. Kharitonov, P. S. Thomas, and P. J. Barnes. 1996. Endogenous nitric oxide is decreased in asthmatic patients by an inhibitor of inducible nitric oxide synthase. *Am. J. Respir. Crit. Care Med.* 154:247–250.
119. Marczin, N., B. Riedel, D. Royston, and M. Yacoub. 1997. Intravenous nitrate vasodilators and exhaled nitric oxide [Letter]. *Lancet* 349:1742.
120. Kharitonov, S. A., G. Lubec, B. Lubec, M. Hjelm, and P. J. Barnes. 1995. L-Arginine increases exhaled nitric oxide in normal human subjects. *Clin. Sci. (Colch).* 88:135–139.
121. Lundberg, J. O., E. Weitzberg, J. Rinder, A. Rudehill, O. Jansson, N. P. Wiklund, J. M. Lundberg, and K. Alving. 1996. Calcium-independent and steroid-resistant nitric oxide synthase activity in human paranasal sinus mucosa. *Eur. Respir. J.* 9:1344–1347.

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122. Garnier, P., I. Fajac, J. F. Dessanges, J. Dall'Ava-Santucci, A. Lockhart, and A. T. Dinh-Xuan. 1996. Exhaled nitric oxide during acute changes of airways calibre in asthma. *Eur. Respir. J.* 9:1134–1138.
123. Silkoff, P., S. Kesten, J. Maurer, P. McClean, H. Furlott, A. S. Slutsky, and N. Zamel. 1995. A technique to minimise the contribution of nasal nitric oxide to exhaled nitric oxide measured at the mouth in humans (abstract). *Am. J. Respir. Crit. Care Med.* 151(Suppl. 2):A329.
124. Takahashi, Y., H. Kobayashi, H. Mitsufuji, and T. Tomita. 1997. [A standard maneuver to measure the concentration of exhaled nitric oxide] [Japanese]. *Nippon Kyobu Shikkan Gakkai Zasshi* 35:596–601.
125. Lundberg, J. O., E. Weitzberg, J. M. Lundberg, and K. Alving. 1996. Nitric oxide in exhaled air. *Eur. Respir. J.* 9:2671–2680.
126. Kharitonov, S. A., B. J. O'Connor, D. J. Evans, and P. J. Barnes. 1995. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 151:1894–1899.
127. Therminarias, A., P. Flore, A. Favre-Juvin, M. F. Oddou, M. Delaire, and F. Grimbert. 1998. Air contamination with nitric oxide: effect on exhaled nitric oxide response. *Am. J. Respir. Crit. Care Med.* 157:791–795.
128. Hyde, R. W., E. J. Geigel, A. J. Olszowka, J. A. Krasney, R. E. Forster, M. J. Utell, and M. W. Frampton. 1997. Determination of production of nitric oxide by lower airways of humans—theory. *J. Appl. Physiol.* 82:1290–1296.
129. Dinarevic, S., C. A. Byrnes, A. Bush, and E. A. Shinebourne. 1996. Measurement of expired nitric oxide levels in children. *Pediatr. Pulmonol.* 22:396–401.
130. Artlich, A., J. U. Hagenah, S. Jonas, P. Ahrens, and L. Gortner. 1996. Exhaled nitric oxide in childhood asthma. *Eur. J. Pediatr.* 155:698–701.
131. Lanz, M. J., D. Y. Leung, D. R. McCormick, R. Harbeck, S. J. Szefler, and C. W. White. 1997. Comparison of exhaled nitric oxide, serum eosinophilic cationic protein, and soluble interleukin-2 receptor in exacerbations of pediatric asthma. *Pediatr. Pulmonol.* 24:305–311.
132. Canady, R. G., T. Platts-Mills, A. Murphy, R. Johannesen, and B. Gaston. 1999. Vital capacity reservoir and online measurement of childhood nitrosopnea are linearly related: clinical implications. *Am. J. Respir. Crit. Care Med.* 159:311–314.
133. Jopsis, Q., S. L. Schellekens, A. Kroesbergen, and J. C. de Jongste. 1998. Detection of nitric oxide in exhaled air in children: validation of the balloon method (abstract). *Am. J. Respir. Crit. Care Med.* 157(3):A469.
134. Baraldi, E., N. M. Azzolin, C. Carra, S. Darlo, R. Ongaro, P. Bipan, and F. Zachello. 1998. Effect of atmospheric nitiric oxide (NO) on measurements of exhaled NO in asthmatic and normal children. *Pediatr. Pulmonol.* 26(1):30–34.
135. Baraldi, E., S. Carra, C. Dario, N. Azzolin, R. Ongaro, G. Marcer, and F. Zacchello. 1999. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. *Am. J. Respir. Crit. Care Med.* 159:262–266.
136. Kissoon, N., P. E. Silkoff, S. Murphy, D. Cancel, K. Blake, and C. Taylor. 1998. Measurement of pulmonary exhaled nitric oxide: relationship to exhalation rates and inline versus bag collection techniques (abstract). *Crit. Care Med.* 26(1):A117.
137. Pfaff, J. K., and W. J. Morgan. 1994. Pulmonary function in infants and children. *Pediatr. Clin. N. Am.* 41:401–423.
138. Baraldi, E., N. M. Azzolin, P. Biban, and F. Zacchello. 1997. Effect of antibiotic therapy on nasal nitric oxide concentration in children with acute sinusitis. *Am. J. Respir. Crit. Care Med.* 155:1680–1683.
139. Baraldi, E., N. M. Azzolin, S. Carra, C. Dario, L. Marchesini, and F. Zachello. 1998. Effect of topical steroids on nasal nitric oxide in children with perennial allergic rhinitis—a pilot study. *Respir. Med.* 92: 558–561.
140. Haight, J. S. J, P. G. Djupesland, W. Qian, H. Furlott, J. Irish, P. McClean, R. Fenton, V. Hoffstein, and N. Zamel. 1999. Does nasal nitric oxide come from the sinuses? *J. Otolarngol.* 28:197–204.
141. Dubois, A. B., J. S. Douglas, J. T. Stitt, and V. Mohsenin. 1998. Production and absorption of nitric oxide gas in the nose. *J. Appl. Physiol.* 84:1217–1224.
142. Cole, P. 1993. Cleansing and conditioning. *In* The Respiratory Role of the Upper Airways: A Selective Clinical and Pathophysiological Review. Mosby Year Book, St. Louis, MO.
143. Djupesland, P. G., J. M. Chatkin, W. Qian, P. McClean, P. Cole, N. Zamel, and J. S. J. Haight. 1999. Aerodynamic influences on nasal nitric oxide output measurement. *Acta Otolaryngol.* 119:479–485.
144. Qian, W., P. J. Djupesland, J. M. Chatkin, P. McClean, H. Furlott, J. S. Chapnik, N. Zamel, and J. S. Haight. 1998. Aspiration flow optimized for nasal nitric oxide measurement. *Rhinology* 37:61–65.
145. Giraud, G. D., B. Nejadnik, B. Kimberly, and W. E. Holden. 1998. Physical characteristics and gas composition of nasal air affect nasal nitric oxide release. *Respir. Physiol.* 114:285–296.
146. Schedin, U., C. Frostell, and L. E. Gustafsson. 1995. Nitric oxide occurs in high concentrations in monkey upper airways. *Acta Physiol. Scand.* 155:473–474.
147. Giraud, G. D., B. Nejadnik, B. Kimberly, and W. E. Holden. 1995. Effects of airflow, temperature or a topical vasoconstrictor on nasal nitric oxide release (abstract). *FASEB J.* 9:A326.
148. Rodenstein, D. O., and D. C. Stanescu. 1983. Absence of nasal airflow during pursed lips breathing. *Am. Rev. Respir. Dis.* 128:716–718.
149. Ferguson, E. A., and R. Eccles. 1997. Changes in nasal nitric oxide concentration associated with symptoms of common cold and treatment with a topical nasal decongestant. *Acta Otolaryngol. (Stockholm)* 117: 614–617.
150. Ferguson, E. A., and R. Eccles. 1997. Relationship between nasal nitric oxide concentration and nasal airway resistance. *Rhinology* 35:120–123.
151. Chatkin, J. M., P. G. Djupesland, W. Qian, P. McClean, H. Furlott, C. Gutierrez, N. Zamel, and J. S. Haight. 1998. Nasal nitric oxide is independent of nasal cavity volume. *Am. J. Rhinol.* 13:179–184.
152. Kase, Y., O. Hilberg, and O. F. Pedersen. 1994. Posture and nasal patency: evaluation by acoustic rhinometry. *Acta Otolaryngol. (Stockholm)* 114:70–74.
153. Rinder, J., J. O. N. Lundberg, A. Anggard, K. Alving, and J. M. Lundberg. 1996. Effects of topical nasal decongestants, L-arginine and nitric oxide synthase inhibition on nasal cavity nitric oxide levels and nasal cavity volume in Man. *Am. J. Rhinol.* 10(6):399–408.
154. Dillon, W. C., V. Hampl, P. J. Shultz, J. B. Rubins, and S. L. Archer. 1996. Origins of breath nitric oxide in humans. *Chest* 110:930–938.
155. Wilkins, J. P., G. D. Giraud, H. A. Mulczuk, and W. E. Holden. 1996. Temperature conditioning of inspired air by the nose—is nitric oxide involved? (abstract). *FASEB J.* 10:A113.
156. Silkoff, P. E., Y. Roth, P. McClean, P. Cole, J. Chapnik, and N. Zamel. 1999. Nasal nitric oxide does not control basal nasal patency or the acute congestion post-allergen challenge in allergic rhinitis. *Ann. Otol. Rhinol. Laryngol.* 108:368–372.
157. van der Mark, T. W., E. Kort, R. J. Meijer, D. S. Postma, and G. H. Koeter. 1997. Water vapour and carbon dioxide decrease nitric oxide readings. *Eur. Respir. J.* 10:2120–2123.
158. Meijer, R. J., H. A. Kerstjens, D. S. Postma, G. H. Koeter, and T. W. van der Mark. 1996. Exhaled nitric oxide concentration is influenced by alcohol containing disinfectants [Letter]. *Eur. Respir. J.* 9:1111.

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